Folin Ciocalteau
Phenolic Content Quantification Assay Kit

KB-03-006

400 tests (96 well plate)

BQCkit

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All chemicals should be handled with care

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- This kit is for R&D use only

Introduction

Polyphenols have become an intense focus of research interest because of their perceived health-beneficial effects. They occur in a variety of fruits, vegetables, nuts, seeds, flowers, bark, beverages, and even some manufactured food, as a component of the natural ingredients used. They have been reported to exhibit anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immunemodulating, anti-microbial, vasodilatory and analgesic effects.

Interest in the research of polyphenols from different natural sources has grown because polyphenols can be utilized as antioxidants in the food industry, and they benefit human health in various ways. The beneficial effects of polyphenols on human health could be due to their free radical scavenger properties, blocking the deleterious action of these molecules on cells.

Phenolic Quantification Assay is based on Folin-Ciocalteu method. The FC reagent contains phosphomolybdic/ phosphotungstic acid complexes. The method relies on the transfer of electrons in alkaline medium from phenolic compounds to form a blue chromophore constituted by a phosphotungstic/phosphomolybdenum complex where the maximum absorption depends on the concentration of phenolic compounds. The reduced Folin-Ciocalteu reagent

is detectable with a spectrophotometer in the range of 690 to 710 nm. The reaction temperature has been used to reduce the time necessary to attain the maximum color (T= 37ºC).

Generally, gallic acid is used as the reference standard compound and results are expressed as gallic acid equivalents (mg/mL).

Materials

BQCkit Phenolic Quantification Assay Kit *KB-03-006* contains:

Product	Quantity	Storage
Reagent A	1 bottle	RT
Reagent B*	1 bottle	RT
Reagent C	1 vial	RT
Standard	2 vials	RT

*If Reagent B is gel-like, it would be necessary to heat it until complete homogenization (if it is necessary, it can be heated up to 55ºC)

Assay Principle

The F-C assay has been used as a measure of total phenolics in natural products, but the basic mechanism is an oxidation/reduction reaction. In the original F-C assay, the carbonate buffer is used for pH adjustment and the end-point of the reaction was attained after 120 min at room temperature, which makes its implementation for routine analysis difficult.

The proposed method was performed in a 96-well microplate format and it was applied to several phenolic compounds and food products (wines, beers, infusions, juices).

Pre-Assay Protocol

1. Dilute the Reagent A in a relation 1: 10 with distilled water in a vial (not included). This dilution will be called RA Working Solution.
2. Add 1.5 mL of Reagent C in each Standard vial. Once dissolved, keep it at -20ºC.
3. Dilute the unknown samples until they reach an absorbance within the limits of the standard curve (See Figure 2 for absorbance values).

Assay Protocol

BQC Phenolic Quantification Kit Microplate

1. Prepare standards containing a range of 0 to 300 µg/mL to a volume of 200 µL.
2. Pipette 20 µL of each standard and unknown samples into a microplate well. Refer to the Table 1 as a guide for diluting the standard. For the diluent, use the same buffer as in the samples.
3. To each well, add 100 µL of RA Working Solution.
4. Add 80 µL of Reagent B to each well.

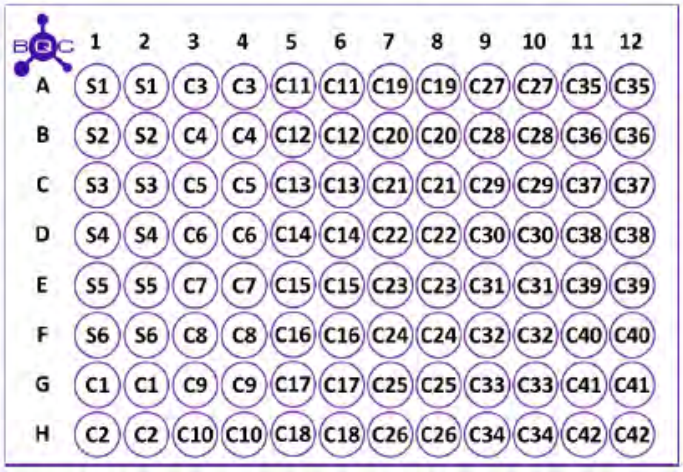
Table 1. *Preparation of diluted standards*

Sample	Standard [µL]	Diluent [µL]	Gallic acid [µg/mL]
S1 (Blank)	---	200	---
S2	5	195	25
S3	10	190	50
S4	20	180	100
S5	40	160	200
S6	60	140	300

5. Measure the absorbance of these standards, blanks and unknown samples at 700 nm (T= 37ºC).

Assay Protocol

Plate set up



	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	C3	C3	C11	C11	C19	C19	C27	C27	C35	C35
B	S2	S2	C4	C4	C12	C12	C20	C20	C28	C28	C36	C36
C	S3	S3	C5	C5	C13	C13	C21	C21	C29	C29	C37	C37
D	S4	S4	C6	C6	C14	C14	C22	C22	C30	C30	C38	C38
E	S5	S5	C7	C7	C15	C15	C23	C23	C31	C31	C39	C39
F	S6	S6	C8	C8	C16	C16	C24	C24	C32	C32	C40	C40
G	C1	C1	C9	C9	C17	C17	C25	C25	C33	C33	C41	C41
H	C2	C2	C10	C10	C18	C18	C26	C26	C34	C34	C42	C42

Figure 1. *96-well plate filling format*

S1-S6 = Standards

C1-C42 = Samples

Attention

- This scheme is just a recommendation of how to perform the assay.
- For optimal results, it is recommended to run the standards and the samples for duplicate, but it is the user´s discretion to do so.
- For another test size, extrapolate the volumes from Table 1.

Data Analysis

1. If the spectrophotometer or microplate reader was not zeroed with the blank, then subtract the average blank value from the standard and unknown sample values.
2. Create a standard curve by plotting A700 nm (y-axis) vs. standard, µg (x-axis). Determine the unknown sample concentration using the standard curve.
3. Standard curve example for microplate assay procedure is shown in Figure 2.

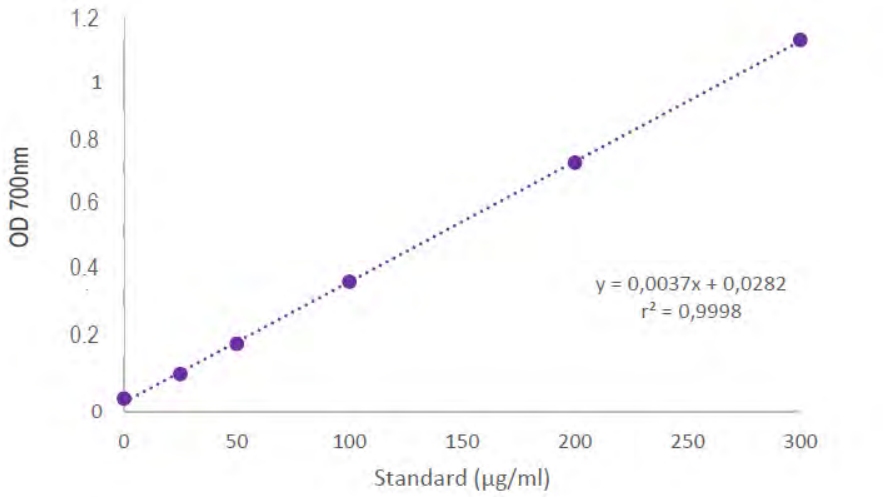


Figure 2. *Typical standard curve using the microplate procedure*

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